2. Lab Methods for Processing Samples

This is the step between collecting and identifying your benthic macroinvertebrate samples. In this part of the process, you separate the critters form the debris in your samples. That process is sometimes referred to as "picking." The result is that you're left with just the organisms you collected, or at least a representative sub-sample, which fits into a few relatively small, labeled vials. You may also preserve the part of the sample left over, which may be just debris, or debris and critters.

Subsampling

The main choice here is whether you will process the whole sample, or just a part of it. Processing just a part of the sample in a systematic way is called "sub-sampling." Sub-sampling must be systematic if you intend to use the results for any estimate of abundance, or any data summaries that require abundance. Estimating the total number of critters in your sample from your sub-sample requires that you pick a standardized portion (e.g. 25%) of the sample, so you can extrapolate from that portion to the whole. If you pick the whole sample, you know the actual abundance. If you sub-sample, you introduce a potential source of error if your sub-sample does not adequately reflect the actual number of critters.

There's a lot of debate among academics and practitioners about the merits of whole versus part of the samples and, if sub-sampling is done, the optimal size of a sub-sample. The issue is, if you sub-sample, how do you assure that your sub-sample is representative? If you are not picking the whole thing, how do you avoid missing critters, especially ones that are small and rare in your sample.

There are two ways to increase the representativeness of your sub-sample:

Randomness: This avoids bias. When subsampling, most people tend to notice the large critters and not the small ones. To avoid this, the sample is typically divided into parts, those parts are randomly selected for sub-sampling, and all the critters in the parts selected are picked.

Sub-sample Size: The more of the sample you pick, the more representative it is. Of course, picking the whole sample would be the most representative Numerous studies have been done which try to find the subsample size at which you get the most representative sample for the fewest organisms picked. They've examined 100 organisms, 300, 1/4 of the sample, 1/2 of the sample, etc. Not all have come to

the same conclusions, but there seems to be general agreement that there is no one “best” way to do it. It depends on the density and diversity of the organisms in your sample.

We include two methods that we feel are good compromises:

Method 2.A.1: 100-critter and 1/4 Sub-sample

One-quarter of the squares in a gridded tray are picked one-by-one directly from the tray. If 100 organism have been picked, then you’re finished. If not, additonal squares in the grid are picked until you reach 100 or more critters. This is the fastest and easiest method, but more prone to errors due to critters drifting around in the tray.

Method 2.A.2: 300-critter, 1/4 Sub-Sample

All the material is removed from the squares in a gridded tray and all the critters picked from the material in 4 of the squares that are randomly chosen. If 300 organism are picked, then you’re finished. If not, material from additional squares in the grid are picked until you reach 300 or more critters. This is somewhat more rigorous and time-consuming, but it avoids errors due to critters drifting around in the tray.

Each of these twop methods combines random selection of the portion of the sample to be picked, and each requires picking a minimum % of the sample from a gridded tray to avoid too small a proportion.

SUBSAMPLING: 100 CRITTTERS AND 1/4 SAMPLE

METHOD **2.A.1**
Lab Processing

This section describes the laboratory procedures for systematically picking and sorting at least 1/4 of the sample and at least 100 organisms: a.k.a a subsample. If used with a semi-quantitative or quantitative sample collection method, this will enable you to use the results for an estimate of abundance, and any data summaries that require abundance.

It is designed so that you can stop after picking the tray and rough sorting into major groups. At some other time, you can do the identification and data analyses.

THE METHOD AT A GLANCE

Sample Type	Whole Field Sample (detritus included)
Sample Preservation	90% Ethyl Alcohol (before adding to sample) or comparable
Sub-sample Type	Random: Minimum of 1/4 of the sample and 100 organisms.
Sub-sampling method	Remove an unbiased, random representative sub-sample by picking critters from a gridded sub-sampling tray with 12 4x4 inch squares.
Minimum % of sample picked	25%

PRELIMINARIES

1) Assemble Processing Equipment and Supplies:

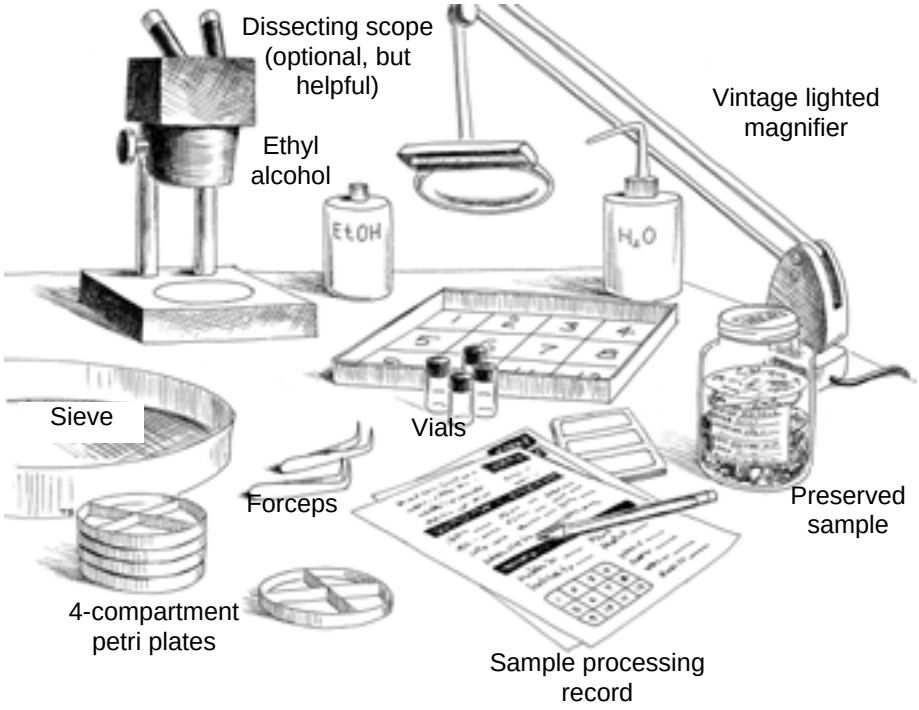
- #30 Sieve (1)
- Labeling Tape & Pencils
- Small Vials (3-4 per sample)
- Lighted Magnifiers (1 per work station)
- One Shallow (1” deep) White Tray
- Four 4-Compartment Petri Plates
- Two Forceps - fine tipped
- Wash bottle w/90% Ethyl Alcohol (1 per work station)
- Sample Processing Record (1 per replicate)
- Random # generator or 12 pieces of paper numbered 1 – 12
- Tally counter (optional)

METHOD 2.A.1
Lab Processing

SUBSAMPLING: 100 CRITTTERS AND 1/4 SAMPLE
SET UP A SAMPLE PROCESSING WORK STATION

Tip:

Instead picking critters out of an open gridded tray, you can buy special trays that come with a gridded framed screen which fits inside the tray. The sample goes on top of the screen and you use a "cookie cutter" type device (which is the size of the squares in the grid) to lift the sample out of each square. Using this device will avoid the drift of organisms into the square you are picking.



This procedure is based on a tray numbered with a 12-square grid. Note that you can use a tray with any number of squares, but it should be seomwhere between 10 and 24 squares.

Step 1: Select the first sample and Inspect it.

Sand/gravel and algae make picking the samples difficult. Sand and gravel clutter the tray and make it difficult to see the organisms. Algae usually has a lot of very small critters entangled. In either case, you need to take special measures to find all the critters.

Is there a lot of algae?

No

Yes

You will need to pick the whole sample.

Is there a lot of sand and gravel?

Yes

Swirl the Sample

See page __

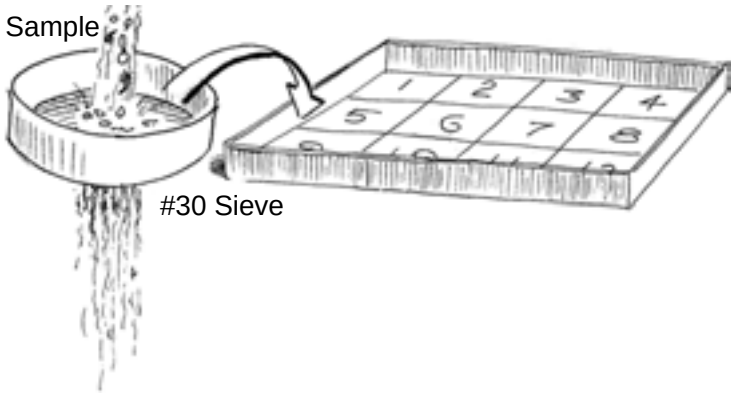
No

Tip:

Organize a team of people at each work station. one person to pick the tray and another person to sort the organisms into tentative major groups and record the information on the Sample Processing Record.

Step 2: Rinse the sample and place it in the tray.

Rinse off and discard large debris like leaves and twigs.



SUBSAMPLING: 100 CRITTTERS AND 1/4 SAMPLE
HOW TO PICK AND SORT A SAMPLE

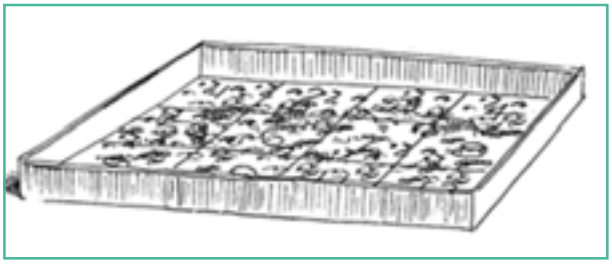
Picking a random starting square:

Use a computer random number generator a printed table.

OR

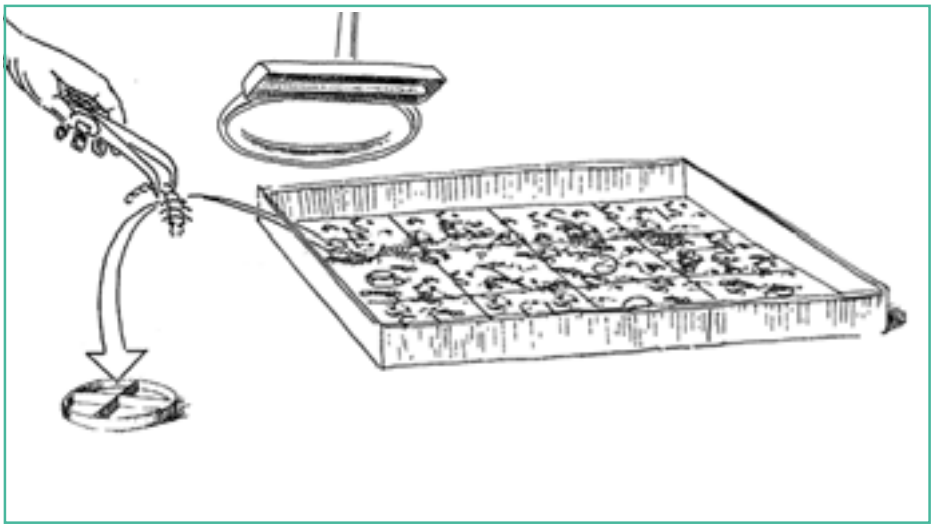
Number twelve pieces of paper from 1 - 12 and place in a container. Pick one of 12 pieces of paper to identify a starting square.

Step 3: Cover the bottom of the tray with about 1/4" of water.and spread the sample evenly over all the squares on the tray.



Step 4: Pick all the organisms from a random starting square and place in the petri plates. Cover them with alcohol.

Mark the bottom of each petri plate with the site and replicate number.



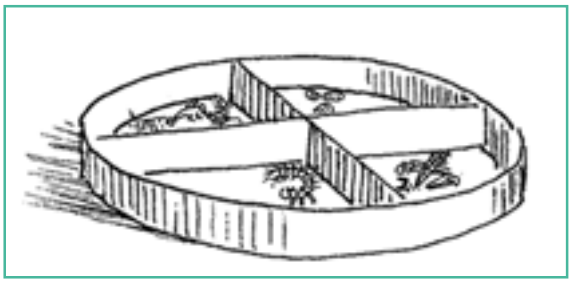
Keep the critters in the petri plates covered with alcohol.

METHOD **2.A.1**
Lab Processing

Step 5: Rough sort the organisms.

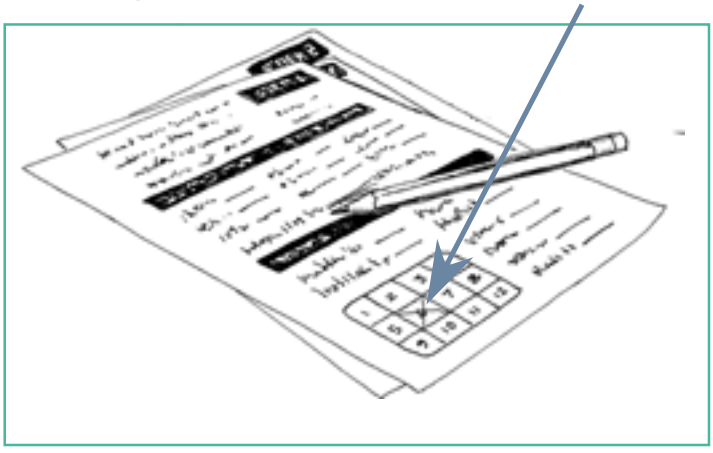
Place similar organisms into the same compartment of petri dish with other similar organisms.

Don't worry about identifying the organisms at this point. Just use the obvious physical differences among the organisms (e.g. overall body shape, # of tails, etc.), It's best not to use too much magnification for the rough sort, but you can use the lighted magnifier or dissecting scope to help you see these differences, but don't worry about precision -- you'll identify them later.



Step 6: Have someone check that there are no critters left in the square when you are finished.

Step 7: As you finish picking squares, mark the corresponding square on the "Sample Processing Record" lab sheet with an "x."



Tips for Picking:

Be sure to look for very small organisms as well as the larger ones.

Turn over rocks, leaves and twigs and look for organisms that may be stuck to these materials.

Pull apart clumps of algae to find organisms that may be tangled there.

Any organism which is lying over a line separating two squares is considered to be in the square containing its head or (if it's headless) the majority of its body.

SUBSAMPLING: 100 CRITTTERS AND 1/4 SAMPLE
HOW TO PICK AND SORT A SAMPLE

Step 8: Select another random square and continue picking and sorting until you've done at least 1/4 of the squares (3 in a 12-square grid) and have over 100 organisms.

If you pick 3 squares and don't yet have 100 organisms, you must continue picking one square at a time until you've picked over 100 organisms. Often, you may have to pick the entire tray.

Be sure to pick all the organisms from the last square, even if you pick well over 100 organisms.

Step 9: Pick rare organisms.

When you have finished picking the squares. Quickly scan the un-picked part of the sample for any types of critters you did not pick from the selected squares. Pick ***one*** of each type. Keep these separate from the rest of the critters.

Step 10: Step 12:Transfer the contents of the petri plate to labeled capped vials filled with alcohol

Transfer the contents of each compartment of the petri plate to its own vial. Place all rare critters that you picked from outside the selected sub-sampled squares in a separate vial labeled "rare."

Fill each vial completely with 90% ethyl alcohol and cap tightly. Using labeling tape and a pencil, label each vial with the site #, replicate #, date, and which vial out of how many total vials (e.g. 4 of 5). Place these labels inside the vials.

SUBSAMPLING: 300 CRITTTERS AND 4 SQUARES

METHOD **2.A.2**
Lab Processing

This section describes the laboratory procedures for systematically picking and sorting at least 1/4 of the sample and at least300 organisms: a.k.a a subsample. If used with a semi-quantitative or quantitative sample collection method, this will enable you to use the results for an estimate of abundance, and any data summaries that require abundance.

This is more rigorous that the previous method and more time-pconsuming, but is less prone to subsampling errors.

It is designed so that you can stop after picking the tray and rough sorting into major groups. At some other time, you can do the identification and data analyses.

THE METHOD AT A GLANCE

Sample Type	Whole Field Sample (detritus included)
Sample Preservation	90% Ethyl Alcohol (before adding to Sample) or comparable
Sub-sample Type	Random: Minimum of 1/4 of the sample and 300 critters
Sub-sampling method	Remove an unbiased, random representative sub-sample using a sub-sample tray or a “Caton” subsampler (outer tray and inner mesh) with 30 6x6 cm squares and “cookie cutter”.
Minimum % of sample picked	25%

PRELIMINARIES

1) Assemble Processing Equipment and Supplies:

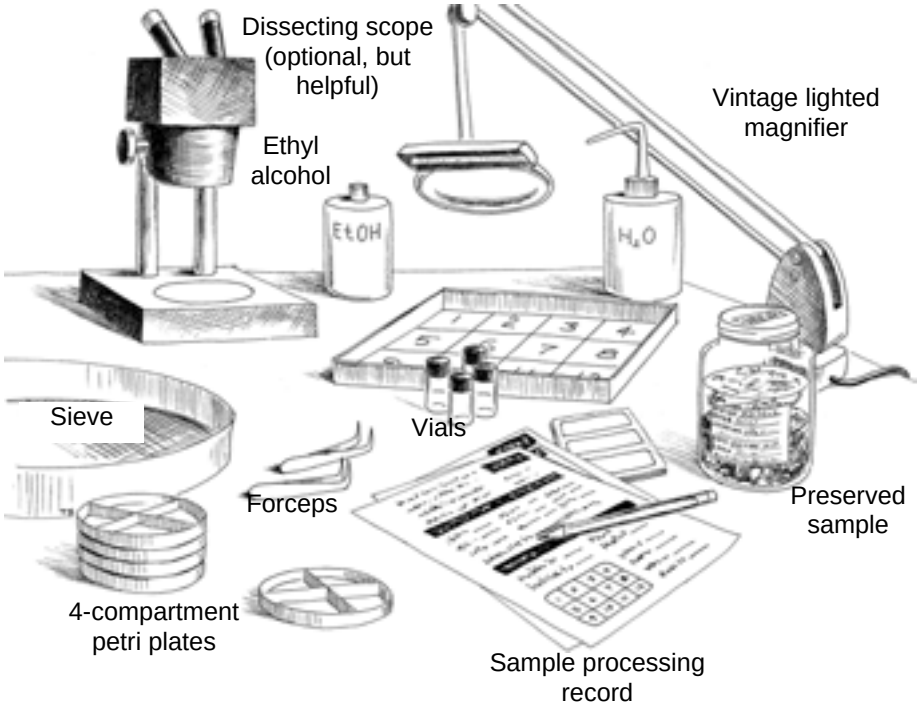
- #30 Sieve (1)
- Labeling Tape & Pencils
- Small Vials (3-4 per sample)
- Lighted Magnifiers (1 per work station)
- One Shallow (1” deep) White Tray or Caton Subsampler
- 2 dozen 4-Compartment Petri Plates
- Two Forceps - fine tipped
- Wash bottle w/90% Ethyl Alcohol (1 per work station)
- Sample Processing Record (1 per replicate)
- Random # generator or 12 pieces of paper numbered 1 – 12
- Tally counter (optional)

METHOD 2.A.2
Lab Processing

SUBSAMPLING: 300 CRITTTERS AND 4 SQUARES
SET UP A SAMPLE PROCESSING WORK STATION

Tip:

Instead picking critters out of an open gridded tray, you can buy "Caton" trays that come with a gridded framed screen which fits inside the tray. The sample goes on top of the screen and you use a "cookie cutter" type device (which is the size of the squares in the grid) to lift the sample out of each square. Using this device will avoid the drift of organisms into the square you are picking.



This procedure is based on a tray numbered with a 12-square grid. Note that you can use a tray with any number of squares, but it should be seomwhere between 10 and 24 squares.

Step 1: Select the first sample and Inspect it.

Sand/gravel and algae make picking the samples difficut. Sand and gravel clutter the tray and make it difficut to see the organisms. Algae usually has a lot of very small critters entangled. In either case, you need to take special measures to find all the critters.

Is there a lot of algae?

No

Yes

You will need to pick the whole sample.

Is there a lot of sand and gravel?

Yes

Swirl the Sample

See page __

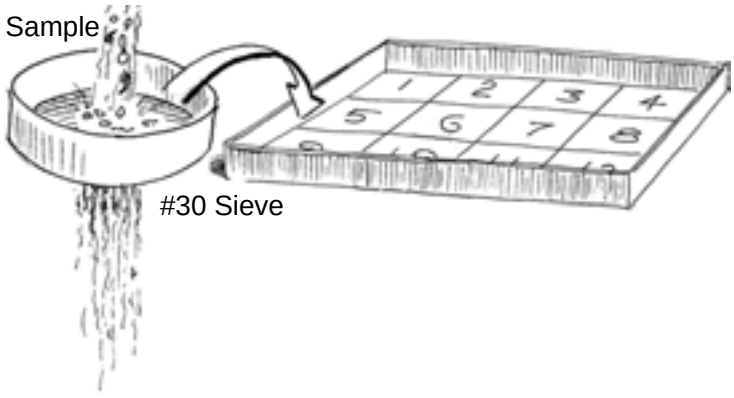
No

Tip:

Organize a team of people at each work station. one person to pick the tray and another person to sort the organisms into tentative major groups and record the information on the Sample Processing Record.

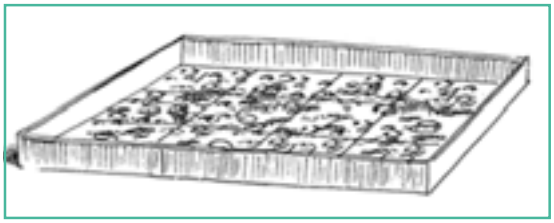
Step 2: Rinse the sample and place it in the tray.

Rinse off and discard large debris like leaves and twigs.



SUBSAMPLING: 300 CRITTTERS AND 4 SQUARES
HOW TO PICK AND SORT A SUB-SAMPLE

Step 3: Spread the sample evenly over all the squares on the tray.



Picking random plates:

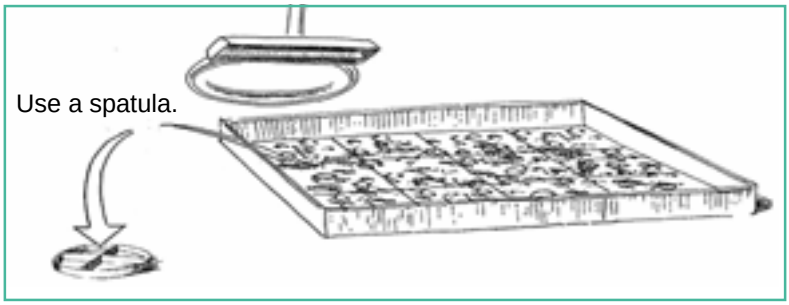
Use a computer random number generator a printed table.

OR

Number twelve pieces of paper from 1 - 12 and place in a container. Pick three of 12 pieces of paper to identify a starting plate.

Step 4: With a spatula, remove all the material from each square and place it in one petri plate for each square. Cover with alcohol.

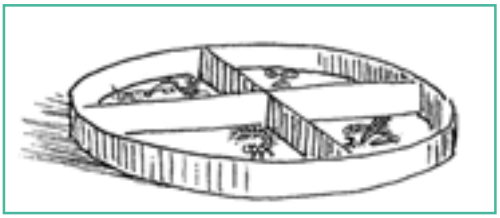
Mark the bottom of each petri plate with the square number, the site and replicate number.



Step 5: Start with a random plate and spread the sample out in a tray. Cover with about 1/4" of water and pick all the critters from the tray and place in a petri plate. Rough sort as you pick into a new 4-compartment petri plate.

Place similar organisms into the same compartment of a new petri plate marked with the site, replicate, and plate #. Mark this plate “rough sort.” Don't worry about identifying the organisms at this point. Just use the obvious physical differences among the organisms (e.g. overall body shape, # of tails, etc.).

SUBSAMPLING: 300 CRITTTERS AND 4 SQUARES
HOW TO PICK AND SORT A SUB-SAMPLE



Keep the critters in the petri plates covered with alcohol.

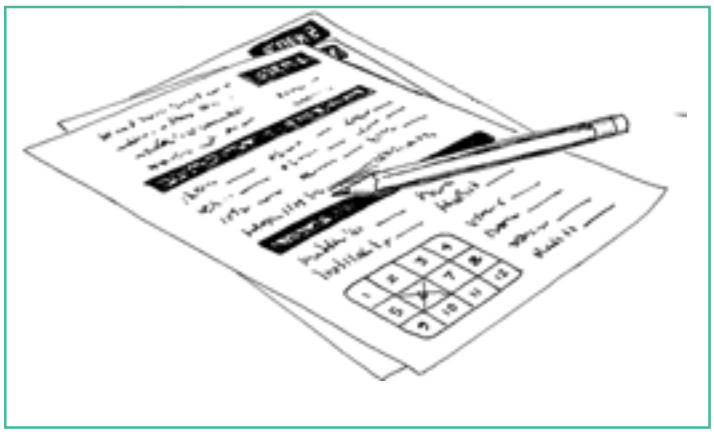
Step 6: Repeat for at least 3 more plates. Stop once you've picked 4 plates, if you have at least 300 organisms. Otherwise, pick one plate at a time until you reach 300. Use the same "rough sort" petri plate for all the critters from the sample.

Pick all the critters from the last plate, even if you go over 300. ***Have someone check that there are no critters left in the tray when you are finished.***

Step 7: Pick rare organisms.

When you have finished picking the plates, quickly scan the un-picked part of the sample in the other plates for any types of critters you did not pick from the selected squares. Pick ***one*** of each type. Keep these separate from the rest of the critters.

Step 8: When you finish picking the plates, mark the corresponding squares on the "Sample Processing Record" lab sheet with an "x."



SUBSAMPLING: 300 CRITTTERS AND 4 SQUARES
HOW TO PICK AND SORT A SUB-SAMPLE

Tips for Picking:

Be sure to look for very small organisms as well as the larger ones.

Turn over rocks, leaves and twigs and look for organisms that may be stuck to these materials.

Pull apart clumps of algae to find organisms that may be tangled there.

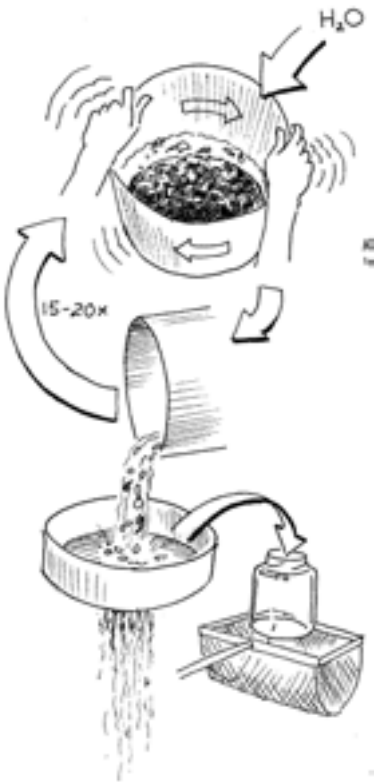
Step 9: Transfer the contents of each the "rough sort" petri plate to labeled capped vials filled with alcohol

Transfer the contents of each compartment of the petri plate to its own vial. Place all rare critters that you picked from outside the selected sub-sampled petri plates in a separate vial labeled "rare."

Fill each vial completely with 90% ethyl alcohol and cap tightly. Using labeling tape and a pencil, label each vialwith the site #, replicate #, date, and which vial out of how many total vials (e.g. 4 of 5)

How To Swirl Samples

Swirling the Sample



1) Place the sample into a #30 sieve and rinse it under tap water. Empty the sieve into a 5-gallon bucket with about double the amount of water it takes to cover the sample.

2) Use a swirling motion to create a whirlpool in the bucket. Organic debris and critters will get caught in the whirlpool.

3) Pour the water and organic debris into a #30 sieve, leaving the rocks, gravel and sand behind on the bottom of the bucket. Leave the material in the sieve.

4) Replace the water in the bucket and repeat the swirling 15-20 times until all that's left in the bucket is rocks, gravel and sand and the water you pour off has no debris in it.

5) Go back to step 3.

About Reference (Voucher) Collections

A reference collection is a set of preserved organisms that are properly identified and labeled. It can be used for a number of things:

Aid to identification: Drawings in keys are intended to clarify the body parts you use to identify the critters. But, they are 2-dimensional and don't always exactly look like the organism in front of you. Real specimens, especially if they are in good shape, can be placed beside the unknown organism.

Record of the types of organisms you've collected: This is sometimes called a "voucher" collection. As a quality control measure, your voucher collection can be used to verify that you've identified the critters in your sample correctly.

Teaching/training tool: Reference collections can be used to train people how to properly identify the critters.

A few suggestions:

* Try to have examples of the critters at different life stages, so you can see how the body parts develop.
* Keep the specimens covered in alcohol. Dried, shriveled critters are no help!
* Try not to handle delicate specimens, like some of the mayflies, too much. Appendages break off easily.
* Make sure that each specimen, when it's removed from the reference collection in a lab session, is transferred to a labeled container and doesn't get mixed in with samples.
* Keep adding fresh specimens as they become available.
* You can buy reference collections from certain biological supply companies. You also may be able to get them donated by an entomology lab.

The main quality assurance challenge is to make sure that all the organisms are correctly identified. This is particularly important when family identification is involved. To assure this, several measures are recommended:

1) Voucher Collection: All processed samples should be saved for later verification by RWN or project staff, the state aquatic biologist (if available) or other professionals. Samples should be stored in labeled vials filled with 90% ethyl alcohol with seals that prevent the alcohol from evaporating. Samples should be checked every few months and the alcohol replenished, if needed.

2) Reference Collection: Examples of each family or major group found should be positively identified by an experienced person. These examples should be stored in vials with a label that correctly identifies the organism. This collection is used to compare with the unknown critters from your river samples to help you identify them.

3) Archive: For various reasons, you may wish to save the picked and/or unpicked art of your samples, in addition to the critters themselves. There are a number of reasons you might want to do this:

4) Picked Debris: As a quality check, you may want an outside professional to go through your picked debris to see if you've missed any critters.

5) Unpicked Debris: If you subampled, you will have left over debris (sand, twigs, etc.) containing critters. You may want to save this material for future processing, for example if your lab processing changes to require picking the whole sample.